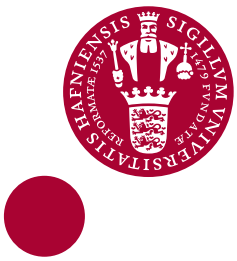

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DEPARTMENT OF DRUG DESIGN AND PHARMACOLOGY
FACULTY OF HEALTH AND MEDICAL SCIENCES
UNIVERSITY OF COPENHAGEN

NMR-based metabolomics for identification of α-amylase inhibitors in rowan berries (*Sorbus* spp.)

Sofie L. Broholm*, Simone M. Gramsbergen*, Nils T. Nyberg, Anna K. Jäger, and Dan Stærk
*Joined first authorship

Introduction

Type 2 diabetes is a metabolic disorder estimated to affect millions of people all over the world.[1] One way of reducing diabetes-related complications is to control postprandial glucose.[2] Inhibition of the carbohydrate digestive enzyme α-amylase is a therapeutic target for maintaining low blood glucose levels. A study from 2011 shows that berries from *Sorbus* spp (rowan berries) effectively inhibit α-amylase activity and suggests that the active compounds are proanthocyanidins.[3] The aim of this project is to identify the rowan berry species with highest α-amylase inhibitory activity - and to find a ^{1}H-NMR method suitable for NMR-based metabolomics.

Collection and in vitro α-amylase assay

16 species of rowan berries were collected in the botanical garden of Copenhagen, Denmark on October 3rd 2014. IC_{50} values of acetone extracts were assessed using a newly developed microplate-based α-amylase inhibition assay.[4]

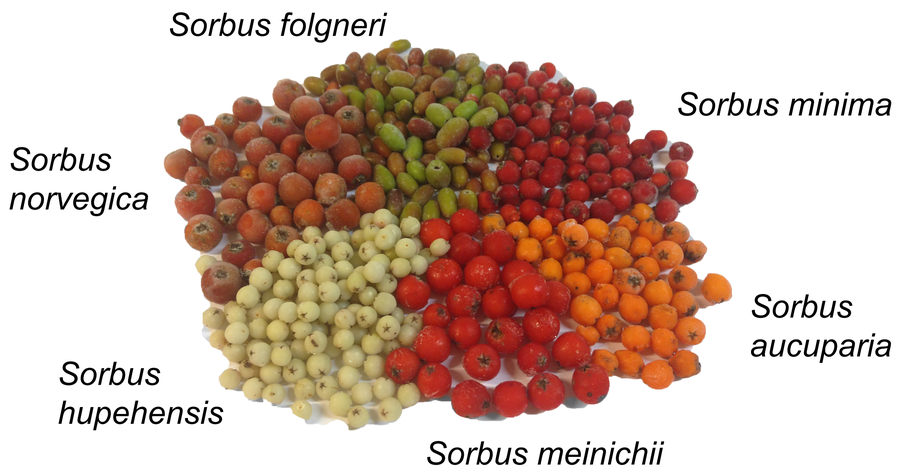

Figure 1: Picture of 6 different species of rowan berries.

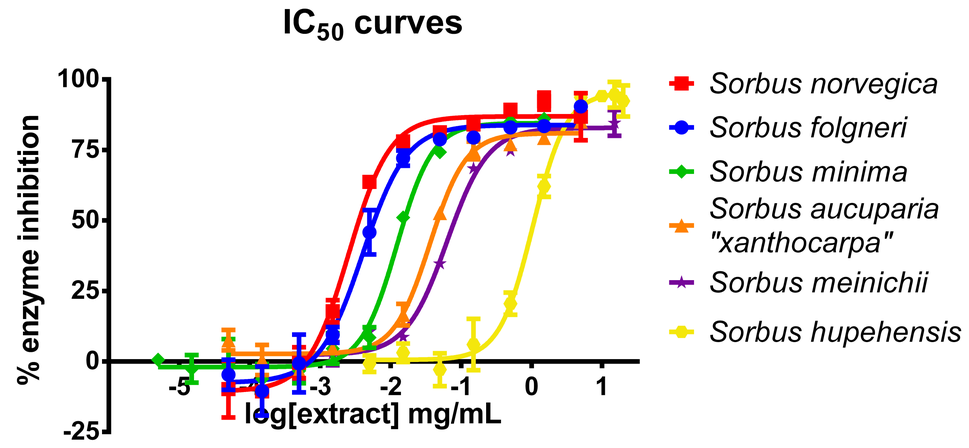

Figure 2: IC_{50} values in (μg/mL): *S. norvegica* = 2.5 ± 0.2; *S. folgneri* = 4.0 ± 0.4; *S. minima* = 12.3 ± 0.9, *S. aucuparia "xanthocarpa"* = 36.2 ± 2.6; *S. meinichii* = 63.2 ± 5.1; *S. hupehensis* = 70

Influence of solvent and temperature on ^{1}H NMR spectra

^{1}H NMR spectra of proanthocyanidins in D_2O at room temperature show very broad ^{1}H-resonances around δ 6-8 ppm due to atropisomerism.[5] *Sorbus folgneri* was therefore analyzed in different solvents and at different temperatures with the aim of obtaining ^{1}H-NMR spectra on the fast exchange time scale.

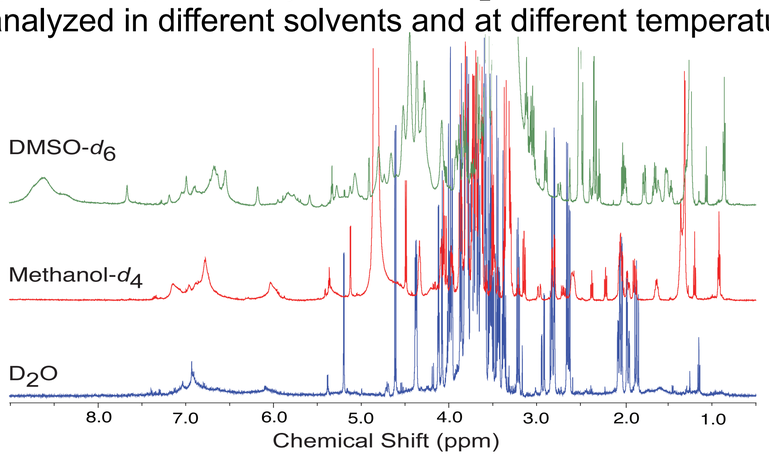

Figure 3: ^{1}H NMR spectra of *Sorbus folgneri* in DMSO-d_6, methanol-d_4 and D_2O.

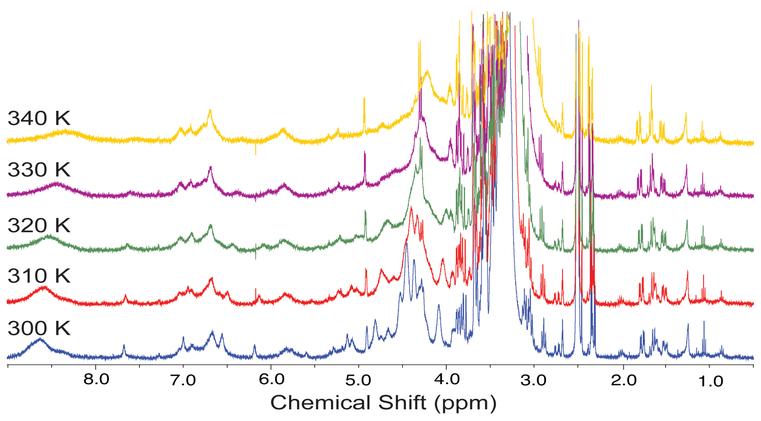

Figure 4: ^{1}H NMR spectra of *Sorbus folgneri* in DMSO-d_6 at temperatures from 300 to 340 K.

Principal components analysis

^{1}H NMR spectra of 16 *Sorbus* spp. were acquired in D_2O phosphate buffer (0.1 M, *p*D 6.0). The PCA-analysis indicates a correlation between the score plot and activity of the extracts due to the scores of component 2 (**Figure 5** and **Figure 6**). The first loading seems to reflect the amount of carbohydrate signals, while the second indicate specific ranges of signals from components most likely related to the α-amylase inhibitory activity.

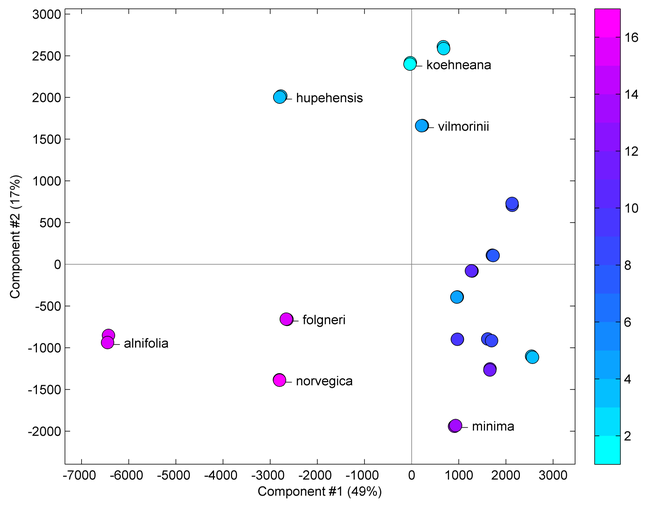

Figure 5: PCA score plot of 16 different *Sorbus* spp. The colors reflect IC_{50}-values. Light pink = most active, while light blue = least active extracts.

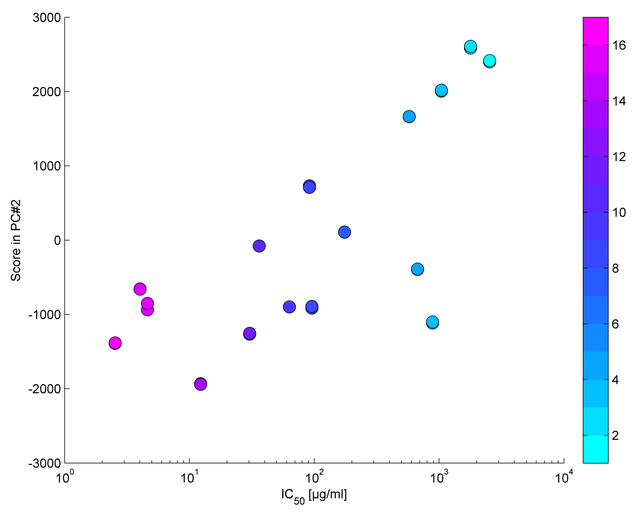

Figure 6: PCA-scores of component 2 vs. IC_{50}-values The colors reflect IC_{50}-values. Light pink = most active, while light blue = the least active extracts.

Concluding remarks and future perspectives

A large variation in α-amylase inhibitory activity of the 16 *Sorbus* spp. was observed in the *in-vitro* α-amylase inhibition assay. Neither solvent or temperature changes in the ^{1}H NMR experiments allowed acquisition at fast exchange. Further experiments could include lowering the temperature to obtain acquisition at slow exchange. The multivariate data analysis score-plot revealed a correlation between the inhibitory effect and finger print of the extracts. This information will be used in our future experiments aiming at identification of components responsible for the α-amylase inhibitory activity in rowan berries.

References

[1]International Diabetes Federation [Internet]. Brussels: IDF Diabetes Atlas; 2014. Available from: http://www.idf.org/diabetesatlas; [2]Marcovecchio ML, Lucantoni M and Chiarelli F. *Diabetes Technol. Ther*. **2011**, *13(3)*, 389-394. [3]Grussu D, Stewart D and McDougall GJ. *J. Agric. Food Chem.* **2011**, *59*, 2324-2331; [4]Okutan L, Kongstad KT, Jäger AK and Staerk D. *J. Agric. Food Chem*. **2014**, *62*,11465-11471; [5]Hümmer W and Schreier P. *Mol. Nutr. Food Res*. **2008**, *52*, 1381-1398.